AD_____

Award Number: DAMD17-98-1-8636

TITLE: Differentially Expressed Genes in Human Prostatic Carcinoma

PRINCIPAL INVESTIGATOR: Jin-Tang Dong, Ph.D.

CONTRACTING ORGANIZATION: University of Virginia
Charlottesville, Virginia 22903

REPORT DATE: August 1999

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 1999	Annual (1 Aug 98 – 31 Jul 99)

4. TITLE AND SUBTITLE
Differentially Expressed Genes in Human Prostatic Carcinoma

5. FUNDING NUMBERS
DAMD17-98-1-8636

6. AUTHOR(S)
Jin-Tang Dong, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Virginia
Charlottesville, Virginia 22903

jd4q@Virginia.EDU

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release;
Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

In spite of the fact that prostate cancer has become a significant health burden, its molecular determinants are poorly understood. We have proposed to identify the genes that are differentially expressed in prostate cancer. One type of such gene is tumor suppressor gene. During the first year of this project, we applied the methods of tissue microdissection and deletion mapping to localize tumor suppressor genes on human chromosome 13. We also used PCR-SSCP and direct sequencing methods to analyze candidate genes for their involvement in prostate cancer. We identified three regions of chromosome 13 for harboring tumor suppressor gene, i.e., 13q14, 13q21, and 13q33. The ERCC5/XPG DNA repair gene at 13q33 was not involved in prostate cancer, neither was the RB1 gene at 13q14. Therefore, each of the three tumor suppressor genes remains to be identified. More interestingly, alterations of 13q14 and 13q21 appeared to be specifically responsible for the aggressive behavior of prostate cancer. Currently, we are performing additional experiments to narrow down the deletion regions that harbor the tumor suppressor genes and started searching for the genes. Identification of these genes will provide targets for improving the diagnosis and therapy of prostate cancer.

14. SUBJECT TERMS
Prostate Cancer, loss of heterozygosity, deletion mapping, tumor suppressor gene, gene mutation

15. NUMBER OF PAGES
19

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

___ Where copyrighted material is quoted, permission has been obtained to use such material.

___ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

___ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

X For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

J. Dony 8-24-99

PI Signature Date

TABLE OF CONTENTS

Page Number

INTRODUCTION:

This project was proposed to identify the genes that are differentially expressed in prostate cancer. Tumor suppressor gene is one type of these genes. Identification of tumor suppressor gene is a significant task in prostate cancer research, because such genes are important targets for improving the diagnosis and treatment of prostate cancer. We first used deletion mapping methods to localize the genes that may be deleted in prostate cancer. Preliminary results have been obtained for the localization of two distinct tumor suppressor genes at the q14 and q21 bands of chromosome 13 whose alterations are responsible for the aggressive behavior of prostate cancer.

BODY:

Our results have been summarized in two papers, one has been published and one is in press. These two papers are:

1. Hyytinen, E. R., Frierson, H. F., Boyd, J. C., Chung, L. W. K., and Dong, J. T. Three distinct regions of allelic loss at 13q14, 13q21-22, and 13q33 in prostate cancer. Genes Chromosomes Cancer, *25:* 108-114, 1999.
2. Hyytinen, E. R., Sipe, T. W., Frierson, H. F., Li, C. L., Degeorges, A., Sikes, R. A., Chung, L. W. K., and Dong, J. T. Loss of heterozygosity and lack of mutations of the *XPG/ERCC5* DNA repair gene in human prostate cancer. Prostate, *in press:* , 1999.

Both of these papers are included in the appendix.

KEY RESEARCH ACCOMPLISHMENTS:

- Chromosome 13 has three distinct regions of LOH at 13q14, 13q21, and 13q33 in prostate cancer.
- The DNA repair gene at 13q33 is not the target gene in prostate cancer.
- The RB1 tumor suppressor gene is not the target gene at 13q14.
- The genes at 13q14 and 13q21 are currently unknown and need to be identified.
- Alterations at 13q14 and 13q21 are responsible for the aggressive behavior of prostate cancer.

REPORTABLE OUTCOMES:

This project has generated two papers, one has been published and one is in press. They are:

1. Hyytinen, E. R., Frierson, H. F., Boyd, J. C., Chung, L. W. K., and Dong, J. T. Three distinct regions of allelic loss at 13q14, 13q21-22, and 13q33 in prostate cancer. Genes Chromosomes Cancer, *25:* 108-114, 1999.

2. Hyytinen, E. R., Sipe, T. W., Frierson, H. F., Li, C. L., Degeorges, A., Sikes, R. A., Chung, L. W. K., and Dong, J. T. Loss of heterozygosity and lack of mutations of the *XPG/ERCC5* DNA repair gene in human prostate cancer. Prostate, *in press:* , 1999.

CONCLUSIONS:

In the first year of this project, we have localized three tumor suppressor genes at 13q14, 13q21, and 13q33 in prostate cancer. The loci at 13q14 and 13q21 appeared to be specifically involved in aggressive prostate cancer, that is particularly important because currently it is more urgent to find molecular markers that can differentiate clinically aggressive prostate cancers from those latent ones. Localization of these genes have validated our efforts to fine map and clone the specific genes involved in the future. In the second year of this project, we will fine map the tumor suppressor genes by homozygous deletion analysis, and start candidate gene searching.

REFERENCES:

See the two papers included in the appendix for the list of references.

Three Distinct Regions of Allelic Loss at 13q14, 13q21-22, and 13q33 in Prostate Cancer

Eija-Riitta Hyytinen,[1,2] Henry F. Frierson, Jr.,[1] James C. Boyd,[1] Leland W.K. Chung,[2] and Jin-Tang Dong[1,3]*

[1]Department of Pathology, University of Virginia Health Sciences Center, Charlottesville, Virginia
[2]Department of Urology, University of Virginia Health Sciences Center, Charlottesville, Virginia
[3]Department of Biochemistry and Molecular Genetics, University of Virginia Health Sciences Center, Charlottesville, Virginia

Chromosome 13 is one of the most frequently altered chromosomes in cancer, including carcinoma of the prostate. Two known tumor suppressor genes, *RB1* and *BRCA2*, map to chromosome 13; however, recent reports suggest that unknown genes on 13q are more likely to be involved in the development of prostate cancer. In order more fully to define the genetic changes on chromosome 13 in prostate neoplasms, we analyzed 27 polymorphic microsatellite markers spanning the q arm for loss of heterozygosity in 40 primary tumors and in metastases from 11 other patients who died of prostate cancer. Of the 40 primary tumors, 23 (58%) showed LOH for at least one marker. Three distinct regions at q14, q21-22, and q33, defined by markers *D13S267* → *D13S153*, *D13S166* → *D13S1225*, and *D13S259* → *D13S274*, showed the most frequent LOH, suggesting their involvement in the development of prostate cancer. For the 12 patients whose tumors showed LOH at these markers, the average age at diagnosis was 58 years, which was younger than that (63 years, $P < 0.05$) for the 28 patients whose tumors lacked LOH. Ten of the 11 (91%) metastases showed LOH with one or more markers. Two of the three most frequently deleted regions (i.e., q14 and q21-22) in the primary tumors and markers linked to the *RB1*, *BRCA2*, and *EDNRB* genes showed high frequencies (56–71%) of LOH in metastases. These results demonstrate that allelic loss on chromosome 13 at q14, q21-22, and q33 occurs in a subset of primary prostate tumors and is a frequent event in metastatic lesions of prostate cancer. *Genes Chromosomes Cancer 25:108–114, 1999.*

INTRODUCTION

The development and subsequent progression of prostate cancer are a complex process requiring multiple genetic abnormalities. An overview of these genetic aberrations has been obtained by comparative genomic hybridization (CGH) and loss of heterozygosity (LOH) analysis (Kunimi et al., 1991; Cher et al., 1994, 1996; Visakorpi et al., 1995; Cunningham et al., 1996). Losses on 6q, 8p, 10q, 13q, 16q, 17p, and 18q are consistently detected, thus providing targets for further studies aimed at identifying the genes residing in these regions. CGH studies have indicated that chromosome 13 is the second most commonly altered chromosome (after chromosome 8) in prostate cancer, showing loss of the q arm in 32% of primary tumors and in 56–75% of recurrent and metastatic tumors. Moreover, each of the four cell lines derived from metastatic prostate cancers, i.e., LNCaP, DU-145, PC-3, and TSU-Pr1, showed loss of 13q sequence with the minimal common deleted region at 13q21 (Nupponen et al., 1998). In addition, loss of chromosome 13 occurs in a number of other cancers such as those of the lung, breast, ovary, and kidney (Yang-Feng et al., 1993; Kuroki et al., 1995; Schoenberg et al., 1995; Maestro et al., 1996; Forozan et al., 1997; Kalachikov et al., 1997; Tamura et al., 1997).

Chromosome 13 harbors the tumor suppressor genes (TSGs) *RB1* and *BRCA2*, which are located at the 13q14.2 and 13q12.2 chromosomal regions, respectively. Allelic loss at the *RB1* locus has been found in about one third of clinically localized prostate tumors (Brooks et al., 1995; Melamed et al., 1997), and mutations of the gene have been detected in a few primary prostate cancers (Kubota et al., 1995). No correlation between LOH and mutation or absence of expression of the *RB1* gene has been observed, however (Cooney et al., 1996; Ittmann and Wieczorek, 1996; Latil et al., 1996). The locus with the most frequent LOH in the q14 region has been found to lie in a 7-cM interval encompassing the loci of *D13S263* and *RB1* (*D13S153*) (Cooney et al., 1996; Ittmann and Wieczorek, 1996; Latil et al., 1996). The *BRCA2* gene has not been found to be frequently altered in primary prostatic neoplasms (Cooney et al., 1996; Ittmann and Wieczorek, 1996; Latil et al., 1996; Melamed et al., 1997).

Supported by: US Army; Grant number: DAMD 17-98-1-8636; American Cancer Society; Grant number: IRG-149L; University of Virginia Cancer Center (Charles E. Myers, Director).

*Correspondence to: Jin-Tang Dong, Ph.D., Department of Pathology, University of Virginia Health Sciences Center, Box 214, Charlottesville, VA 22908. E-mail: jd4q@virginia.edu

Received 28 September 1998; Accepted 31 December 1998

The results of CGH and LOH studies suggest that unidentified genetic loci on 13q are involved more frequently in the development of prostate cancer. No detailed deletion maps using multiple markers that span the entire 13q have been constructed, however. We performed LOH analysis using 27 polymorphic microsatellite markers spanning the q arm of chromosome 13 in 40 primary prostate cancers and in metastases from 11 other patients who died of prostate cancer. Three regions defined by the markers *D13S267* → *D13S153*, *D13S166* → *D13S1225*, and *D13S259* → *D13S274* at q14, q21-22, and q33, respectively, were identified as having the most frequent LOH in a subset of prostate cancers; two of these LOH regions were lost even more frequently in prostate cancer metastases.

MATERIALS AND METHODS

Tumor Samples

Forty pairs of matched zinc formalin-fixed, paraffin-embedded normal and primary prostate cancer tissues from prostatectomy specimens from previously untreated patients were used in this study. Patient ages ranged from 42 to 74 years (median, 60 years). The pathologic characteristics of the tumors were as follows: Gleason score: 82% had scores of 5 to 7, whereas 18% had scores of 8 or 9; lymph node metastases: one of 40 cases; seminal vesicle invasion: 83% negative and 17% positive; capsule penetration: 64% negative and 36% positive. The tumors were staged pathologically according to the most recent AJCC recommendations (AJCC, 1997). Two thirds were pT2 and one third were pT3 cancers. In addition, 11 metastatic cancer specimens from lymph nodes, liver, or bone and matched non-neoplastic tissues from patients who had died of prostate cancer were obtained at autopsy. Tumors were zinc formalin-fixed and paraffin-embedded, and the cells for DNA isolation were collected from 7-μm H- and E-stained sections using a previously described protocol for preparation of histologic sections on glass slides prior to microdissection (Moskaluk and Kern, 1997). Tumor samples were microdissected to ensure a minimum number of 70% neoplastic cells. Non-neoplastic cells from lymph nodes or seminal vesicles (or spleen for autopsy specimens) in almost 90% of the cases, and from normal prostate in the remainder, were obtained from paraffin blocks that contained no neoplastic cells.

LOH Analysis

DNA was isolated from specimens by adding proteinase K solution, incubating at 55°C for 2–3 days, extracting with phenol and chloroform, and precipitating with ethanol. Twenty-seven microsatellite markers, which were either purchased from Research Genetics (Huntsville, AL) or synthesized by Life Technologies (Rockville, MD), were used. The chromosomal location of these markers and their genetic distances (cM) from the top of chromosome 13 have been established as follows (Hudson et al., 1995): *D13S217* (q12.1, 19), *D13S267* (q12.3, 28.9), *D13S263* (q14.1-14.2, 40), *D13S1227* (q14.1-14.2, 41.7), *D13S153* (q14.2, 47.5), *D13S119* (q14.3-21, 51.5), *D13S312* (q21.1-21.3, N/A), *D13S134* (q21.2-22, N/A), *D13S166* (q21.3-22, 57.3), *D13S156* (q21.3-22, 57.3), *D13S313* (q21.1-21.3, N/A), *D13S269* (q21.3-22, 58), *D13S162* (q22, 60), *D13S1306* (q22, 61), *D13S1225* (q22, 64.3), *D13S264* (q22, 65.5), *D13S317* (q22, 66), *D13S154* (q31, 77.1), *D13S121* (q31, N/A), *D13S159* (q32, 81.5), *D13S1240* (q32, 83.7), *D13S259* (q33, 86.9), *D13S158* (q33, 86.9), *D13S280* (q33, 87.5), *D13S274* (N/A, 89.4), *D13S173* (q33-34, 96), and *D13S285* (q34, 113). Three of the markers were physically mapped either at or near known genes. *D13S267* is linked to the *BRCA2* gene (Wooster et al., 1995), *D13S153* is from intron 2 of the *RB1* gene (Toguchida et al., 1993), and *D13S317* is located in a YAC clone that harbors the *EDNRB* gene (Puffenberger et al., 1994).

For the 11 prostate cancer metastases, 14 markers that had LOH at different frequencies (most with high rates, some with lower rates) in informative localized tumors were analyzed in addition to the markers linked to the known gene loci. The sequences of PCR primers for these markers were obtained from the Genome Database (http://gdbwww.gdb.org) and the Center for Genome Research at the Whitehead Institute for Biomedical Research (http://www-genome.wi.mit.edu). Each PCR analysis was performed on 10–25 ng of genomic DNA; 1 × PCR buffer (1.5 mM $MgCl_2$); 0.4 μM of each primer; 200 μM dCTP, dGTP, and dTTP; 5 μM dATP; 2.5 μCi of α-^{35}S-dATP (1,000 mCi/mmol); and 1 U of Taq DNA polymerase in a volume of 10 μl. Thirty-five to 40 cycles, each consisting of denaturation at 94°C for 30 sec, annealing at 48–57°C for 30 sec, and extension at 72°C for 1 min, were performed.

PCR products were separated in a 6% denaturing polyacrylamide gel and autoradiographed for 2–5 days using Kodak BioMax MR films. Allelic loss was determined when the signal for one allele in the tumor was reduced significantly when compared to that for the non-neoplastic cells. The change in the size of an allele in a cancer tissue

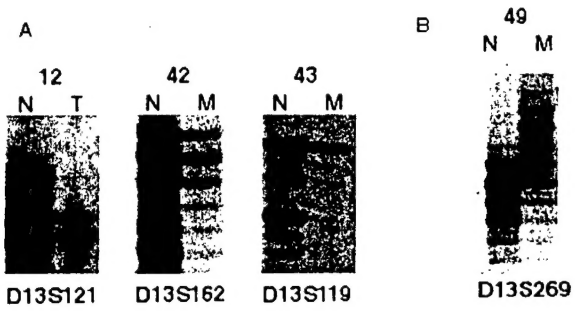

Figure 1. Examples of allelic loss (**A**) and microsatellite instability (**B**) in prostate cancer obtained with microsatellite markers of chromosome 13. N, T, and M indicate normal and matched tumor or metastatic tissue, respectively, for four cases. Case number is on the top.

specimen compared to its matched non-neoplastic tissue sample was termed microsatellite instability (MSI). Examples of microsatellite alterations, which include LOH and MSI, are shown in Figure 1 for markers on chromosome arm 13q. All autoradiograms were analyzed independently by two of the investigators (E.-R.H. and J.-T.D.), and any conflicts in interpretation were resolved by repetition of the experiment.

The relationship between LOH at loci of *D13S263-D13S1227*, *D13S313-D13S269-D13S162*, and *D13S158-D13S280* and patient age at diagnosis, Gleason score, or tumor stage was analyzed statistically by use of the Wilcoxon sign rank test.

RESULTS

Primary Tumors

Of the 40 primary tumors analyzed, 23 (58%) had LOH for at least one marker. LOH at the 27 loci for the primary tumors ranged from 0 to 27% (Fig. 2). Whereas most markers showed LOH in less than 5% of tumors, markers *D13S263*, *D13S313*, *D13S269*, and *D13S158* had allelic loss in at least 14% of informative cases. These markers defined three distinct regions. The most centromeric region (18.6 cM) was at 13q14.2 and was defined by markers *D13S267* and *D13S153*, because markers *D13S263* and *D13S1227* showed LOH in 5/33 cases (15%) and 3/28 (11%) of informative cases, respectively, and 3 tumors had LOH at both of these markers (Fig. 2). This region seems to be different from that of *RB1* (*D13S153*) at 13q14, because none of the tumors with LOH for *D13S263* and *D13S1227* showed LOH at *RB1*. In addition, *RB1* showed LOH in only 1/26 (4%) informative cases. The intermediate region (7 cM) was defined by markers *D13S166* and *D13S1225* at 13q21-22, as *D13S313*, *D13S269*, *D13S162*, and *D13S1306* together showed LOH in seven tumors. The telomeric region (2.5 cM) was at 13q33 and lay between markers *D13S259* and *D13S274*, because five tumors showed LOH at either *D13S158* or *D13S280* or both, whereas alleles were either retained or uninformative for the flanking markers *D13S259* and *D13S274* (Fig. 2). There were 12 tumors that had LOH in at least one of the markers from the three LOH regions. These tumors had a mean Gleason score of 7 and occurred in patients whose age at diagnosis averaged 58 years; 50% of them had a higher local stage (pT3) at prostatectomy. The 28 tumors without LOH at these regions also had intermediate Gleason scores and occurred in patients with an average age at diagnosis of 63 years; 32% of them occurred at stage pT3 (Table 1). The difference in age at diagnosis between these two groups was statistically significant ($P < 0.05$).

The primary cancers with the most frequent microsatellite alterations (including both LOH and MSI) were cases 6, 12, 13, 19, 21, and 25, in which alterations were detected in 6/20 (30%), 5/18 (28%), 7/15 (47%), 3/14 (21%), 7/22 (32%), and 6/19 (32%) of the informative loci respectively. Most of the microsatellite alterations for these six tumors were at the loci of *D13S263*, *D13S313*, *D13S269*, *D13S162*, and *D13S158*. Except for case 13, all the other five cases were among the 12 tumors showing LOH at the three regions most frequently lost. MSI was detected sporadically in 15 (38%) of the tumors. Only one tumor (case 13) appeared to have a replication error phenotype, because each of the allelic alterations was an MSI. The *D13S162* locus from the q22 region showed MSI in nine of the 28 informative cases (32%).

Metastatic Lesions

For the metastases from the 11 patients at autopsy, we analyzed 14 microsatellite markers that were located on different bands of chromosome 13. Ninety-one percent (10/11) of the cases showed LOH with at least one marker (Fig. 3). Markers *D13S263*, *D13S313*, and *D13S269*, which had the highest frequency of LOH in the primary tumors, were also among the markers that had high frequencies (56–71%) of LOH in the metastases. In addition, the three loci linked to the *BRCA2*, *RB1*, and *EDNRB* genes (*D13S267*, *D13S153*, and *D13S317*) were also among the loci with high rates of LOH in the metastases (71, 56, and 56%, respectively). Similar to the findings in the primary tumors, most of the alterations for marker *D13S162* in metastases were scored as MSI rather than LOH. Five of the 11 metastases (cases 42, 43, 44, 46, and 47) showed extensive (>75%) allelic alterations (4 with LOH

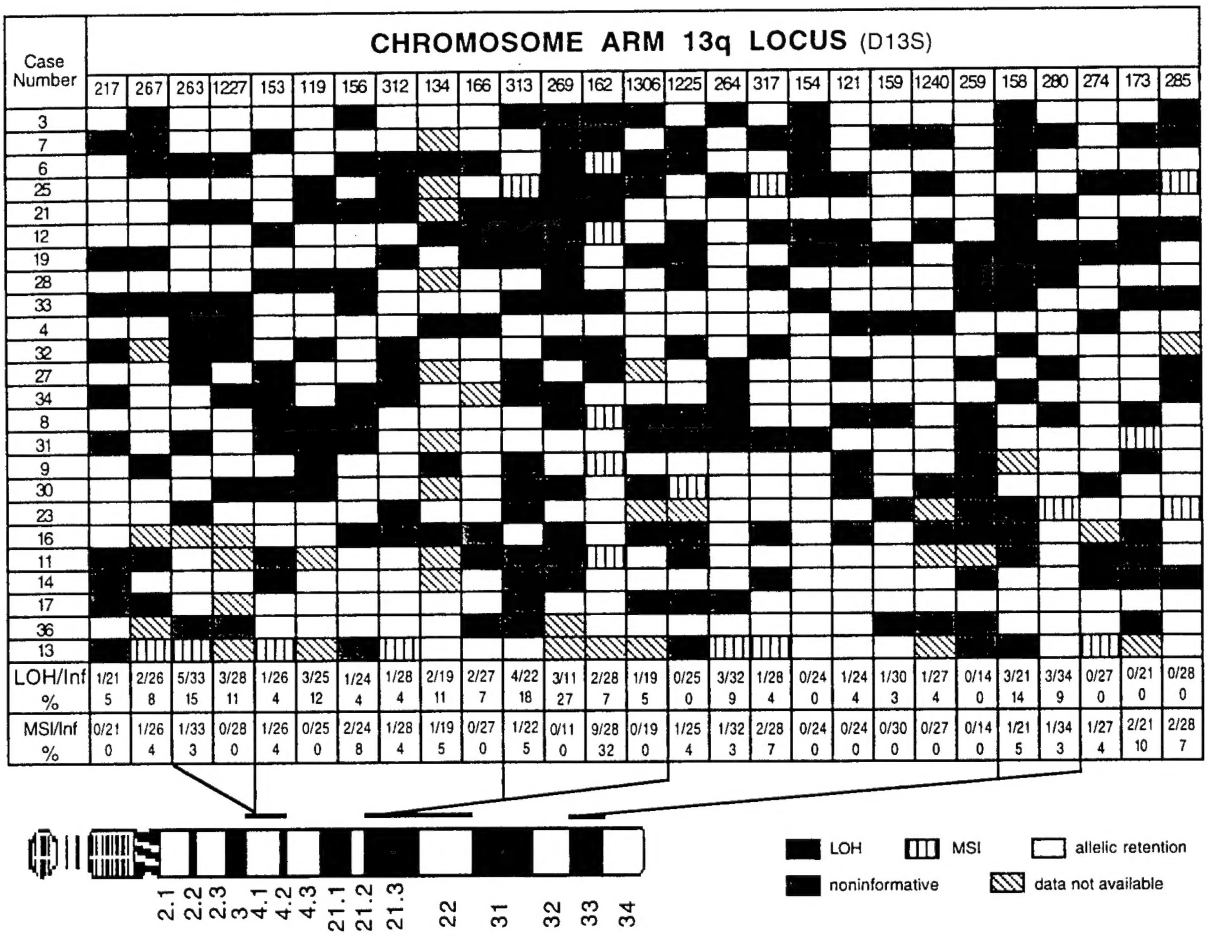

Figure 2. Results of microsatellite analysis for chromosome 13 in primary prostate tumors. Marker numbers are listed at the top, and case numbers are on the left. Only tumors showing LOH or MSI are illustrated. Frequencies of total loss of heterozygosity (LOH) and microsatellite instability (MSI) in informative cases (Inf) for each of the 27 microsatellite markers for all 40 primary tumors are listed at the bottom. The three distinct LOH regions are indicated by bars above the ideogram of chromosome 13.

and one with MSI) at the informative loci. Two of them (cases 42 and 46) had apparently lost the entire chromosomal arm, because all informative loci showed LOH. In addition, two of the 11 tumors (cases 44 and 49) showed MSI for at least 40% of the markers, presenting a replication error phenotype.

DISCUSSION

CGH studies have demonstrated that loss of 13q occurs commonly in prostate cancer. In order to define the distribution and extent of 13q loss, we performed deletion mapping for prostate cancer by using 27 microsatellite markers encompassing the entire q arm. In primary tumors, allelic loss for at least one marker was found in 23 (58%) cases. Half of all LOH was detected at six markers (*D13S263*, *D13S313-D13S269-D13S162*, and *D13S158-D13S280*), which are derived from three regions at q14, q21-22, and q33, respectively.

We found that the primary tumors that had LOH in at least one of the three regions were diagnosed in younger patients and slightly more frequently were of a higher pathologic stage at prostatectomy than were those tumors lacking LOH at these loci. Thus, genetic abnormalities in these chromosomal regions may define a subset of clinically significant primary tumors. Younger age at diagnosis and higher stage are also characteristics of familial prostate cancer (Gronberg et al., 1997). It is unknown, however, whether the 13q loci identified in this study are also important in the development of hereditary prostate cancer.

Frequently deleted loci at 13q covering the potential TSG regions in this study have been found in B-cell chronic lymphocytic leukemia (Kala-

TABLE 1. Clinicopathologic Features of 40 Primary Prostate Tumors and Frequencies of Loss of Heterozygosity (LOH) and Microsatellite Instability (MSI) in Informative (Inf) Cases

Case no.	LOH/ Inf	%	MSI/ Inf	%	Gleason score	Age	Stage
1	0/18	0	3/18	17	7	60	pT3a
2	0/18	0	2/18	11	5	74	pT2b
3	1/18	6	0/18	0	6	47	pT3b
4	3/22	14	0/22	0	7	49	pT2b
5	0/17	0	0/17	0	6	72	pT2b
6	5/20	25	1/20	5	6	60	pT3a
7	0/13	0	1/13	8	7	71	pT3b
8	1/16	6	1/16	7	6	42	pT2b
9	2/21	10	1/21	5	6	60	pT3b
10	0/14	0	1/14	7	7	64	pT2
11	1/13	8	1/13	8	5	61	pT2a
12	4/18	22	1/18	6	6	56	pT2b
13	0/15	0	7/15	47	8	68	pT3b
14	1/18	6	0/18	0	8	69	pT2b
15	0/12	0	0/12	0	7	62	pT2
16	1/12	8	0/12	0	8	60	pT2b
17	1/21	5	0/21	0	7	59	pT3b
18	0/17	0	0/17	0	7	65	pT2b
19	3/14	21	0/14	0	5	50	pT2b
20	0/18	0	0/18	0	6	57	pT2b
21	7/22	32	0/22	0	8	59	pT2a
22	0/13	0	0/13	0	7	62	pT2b
23	1/17	6	2/17	12	6	67	pT2b
25	3/19	16	3/19	16	7	69	pT3b
26	0/14	0	1/14	8	7	61	pT2b
27	1/16	6	0/16	0	9	53	pT3b
28	1/18	6	0/18	0	9	65	pT3
29	0/19	0	0/19	0	6	59	pT2b
30	1/17	6	1/17	6	6	57	pT3a
31	1/16	6	1/16	7	5	67	pT2b
32	2/17	12	0/17	0	7	60	pT2b
33	1/15	7	0/15	0	7	56	pT2b
34	2/18	11	0/18	0	6	65	pT2b
35	0/18	0	0/18	0	7	70	pT3a
36	1/19	5	0/19	0	6	57	pT2a
37	0/14	0	0/14	0	6	55	pT2b
38	0/17	0	1/17	6	6	65	pT2b
39	0/14	0	0/14	0	7	70	pT3a
40	0/14	0	0/14	0	8	70	pT3a
41	0/17	0	0/17	0	7	56	pT3

chikov et al., 1997) as well as in cancers of the lung (Tamura et al., 1997), head and neck (Maestro et al., 1996), liver (Kuroki et al., 1995), kidney (Schoenberg et al., 1995), and ovary (Yang-Feng et al., 1993). In some cancers, deletion of these loci has been associated with a poor prognosis (van den Berg et al., 1996), aggressive behavior (Dotzenrath et al., 1996), and tumor recurrence (Yamaguchi et al., 1996). It is possible that the loci identified in this study are also involved in the development and progression of other cancers.

Our results indicate that allelic loss at the 13q14 region is centered at *D13S263-D13S1227* rather than at the *RB1* locus. The *D13S263* locus, which is 7 cM to the *RB1*, is located in a region that has shown frequent LOH in previous studies of prostate cancer (Cooney et al., 1996; Ittmann and Wieczorek, 1996; Latil et al., 1996). Therefore, although the *RB1* gene seems to play a role in some prostate cancers, another TSG may be located at 13q14.

Based on a recently constructed high-resolution YAC-cosmid-STS map, the *ERCC5/XPG* gene is in the telomeric LOH region, because markers *D13S280* and *D13S158* and this gene were located to the same DNA fragment of 1 megabase (Cayanis et al., 1998). *ERCC5/XPG* is a DNA excision repair gene whose mutations have been detected in patients with xeroderma pigmentosum group G (Nouspikel and Clarkson, 1994). To determine its possible role in prostate cancer, we are currently performing mutation analysis for tumors that showed loss of the telomeric region.

In CGH analyses, 13q loss was observed in 32% of primary tumors, 75% of metastatic lesions, and each of four prostate cancer cell lines. Our results for metastases are in agreement with these CGH findings, as 10/11 (91%) of the cases showed LOH with at least one marker, compared to 23/40 (58%) for primary tumors. Allelic loss at 13q seems to be a frequent event in metastases. It is unknown, however, whether 13q loss is of biological significance and serves as a selection force driving tumor cells toward aggressive behavior, or is simply a result of genetic instability. In the study of Li et al. (1998), the rate of allelic loss at 13q12-q14 was slightly lower in metastases compared to localized tumors. One possible explanation for this inconsistency is that the loci analyzed were different among these studies.

Regions of the *BRCA2*, *RB1*, and *EDNRB* genes, which showed LOH in 8% or fewer of the primary tumors, showed LOH in 56–71% of the metastases. Hence, inactivation of these genes may be more important in metastatic lesions than in primary tumors of prostate cancer. This conclusion is supported by previous studies, in which the *RB1* gene had frequent LOH or loss of expression in aggressive prostate cancers (Melamed et al., 1997; Theodorescu et al., 1997). The *BRCA2* gene had frequent LOH in advanced prostate cancers, and some men with germline *BRCA2* mutations developed and died of prostate cancer (Sigurdsson et al., 1997). Also, a mutation of the *EDNRB* gene occurred in the LNCaP metastatic prostate cancer cell line (Nelson et al., 1998). Three markers at 13q14.1-14.2 and 13q21.1-22 that showed the highest rate of

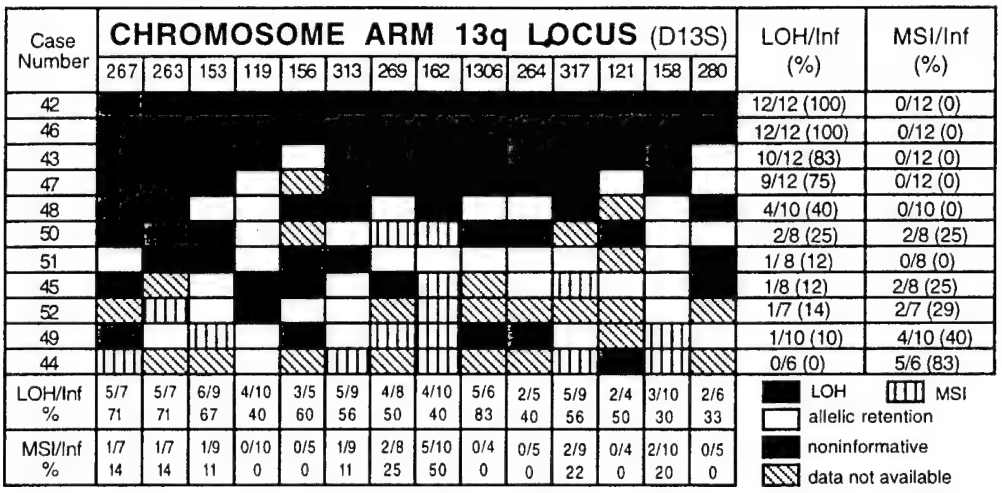

Figure 3. Results of microsatellite analysis for chromosome 13 in metastatic prostate cancer. Marker numbers are listed at the top, and case numbers are on the left. Frequencies of loss of heterozygosity (LOH) and microsatellite instability (MSI) in informative cases (Inf) are listed at the bottom for each of the 14 microsatellite markers and on the right for each of the metastases.

LOH in primary tumors showed an even higher (56–71%) frequency of allelic loss in the metastatic lesions, suggesting that these regions of chromosome 13 harbor genes that are important to both primary and highly aggressive, metastatic prostate cancers.

Along with 13q, chromosomes arms 8p and 16q have shown more frequent LOH in metastases than in primary tumors of prostate cancer. In previous studies, 83% and 61 to 73% of metastatic lesions showed LOH for 8p and 16q (Trapman et al., 1994; Suzuki et al., 1996; Pan et al., 1998), respectively, which are comparable to the LOH rate (91%) for chromosome arm 13q in our study. These data suggest that these chromosomes contain genes associated with advanced prostate cancer.

The frequency of microsatellite instability in prostate cancer has been variable in previous studies (Terrell et al., 1995; Uchida et al., 1995; Watanabe et al., 1995; Cunningham et al., 1996). Our results are consistent with two of the studies in which MSI was detected in about half of the primary tumors studied. However, replication error phenotype due to defective mismatch repair is considered to be present only if at least one third of the examined loci exhibit MSI (Honchel et al., 1995). Based upon this definition, one of the 40 primary tumors and two of the 11 metastatic cancers showed a replication error phenotype. Thus, altered DNA mismatch repair may function in advanced prostate cancer.

In summary, our findings show that, in prostate cancer, the most frequent losses on chromosome 13 are located at 13q14, 13q21-22, and 13q33. Allelic loss at these regions was associated with younger age at diagnosis and might define a subgroup of clinically significant prostate cancer.

REFERENCES

AJCC. 1997. AJCC cancer staging manual, 5th ed. Philadelphia: Lippincott-Raven.

Brooks JD, Bova GS, Isaacs WB. 1995. Allelic loss of the retinoblastoma gene in primary human prostatic adenocarcinomas. Prostate 26:35–39.

Cayanis E, Russo JJ, Kalachikov S, Ye X, Park SH, Sunjevaric I, Bonaldo MF, Lawton L, Venkatraj VS, Schon E, Soares MB, Rothstein R, Warburton D, Edelman IS, Zhang P, Efstratiadis A, Fischer SG. 1998. High-resolution YAC-cosmid-STS map of human chromosome 13. Genomics 47:26–43.

Cher ML, MacGrogan D, Bookstein R, Brown JA, Jenkins RB, Jensen RH. 1994. Comparative genomic hybridization, allelic imbalance, and fluorescence in situ hybridization on chromosome 8 in prostate cancer. Genes Chromosomes Cancer 11:153–162.

Cher ML, Bova GS, Moore DH, Small EJ, Carroll PR, Pin SS, Epstein JI, Isaacs WB, Jensen RH. 1996. Genetic alterations in untreated metastases and androgen-independent prostate cancer detected by comparative genomic hybridization and allelotyping. Cancer Res 56:3091–3102.

Cooney KA, Wetzel JC, Merajver SD, Macoska JA, Singleton TP, Wojno KJ. 1996. Distinct regions of allelic loss on 13q in prostate cancer. Cancer Res 56:1142–1145.

Cunningham JM, Shan A, Wick MJ, McDonnell SK, Schaid DJ, Tester DJ, Qian J, Takahashi S, Jenkins RB, Bostwick DG, Thibodeau SN. 1996. Allelic imbalance and microsatellite instability in prostatic adenocarcinoma. Cancer Res 56:4475–4482.

Dotzenrath C, Teh BT, Farnebo F, Cupisti K, Svensson A, Toell A, Goretzki P, Larsson C. 1996. Allelic loss of the retinoblastoma tumor suppressor gene: a marker for aggressive parathyroid tumors? J Clin Endocrinol Metab 81:3194–3196.

Forozan F, Karhu R, Kononen J, Kallioniemi A, Kallioniemi OP. 1997. Genome screening by comparative genomic hybridization. Trends Genet 13:405–409.

Gronberg H, Isaacs SD, Smith JR, Carpten JD, Bova GS, Freije D, Xu J, Meyers DA, Collins FS, Trent JM, Walsh PC, Isaacs WB. 1997. Characteristics of prostate cancer in families potentially linked to the hereditary prostate cancer 1 (HPC1) locus. JAMA 278:1251–1255.

Honchel R, Halling KC, Thibodeau SN. 1995. Genomic instability in neoplasia. Semin Cell Biol 6:45–52.

Hudson TJ, Stein LD, Gerety SS, Ma J, Castle AB, Silva J, Slonim DK, Baptista R, Kruglyak L, Xu SH, Hu X, Colbert AME, Rosenberg C, Reeve-Daly MP, Rozen S, Hui L, Wu X, Vestergaard C, Wilson KM, Bae JS, Maitra S, Ganiatsas S, Evans CA, DeAngelis MM, Ingalls KA, Nahf RW, Horton LT, Anderson MO, Collymore AJ, Ye W, Kouyoumjian V, Zemsteva IS, Tam J, Devine R, Courtney DF, Renaud TM, Nguyen H, O'Connor TJ, Fizames C, Faure S, Gyapay G, Dib C, Morissette J, Orlin JB, Birren BW, Goodman N, Weissenbach J, Hawkins TL, Foote S, Page DC, Lander ES. 1995. An STS-based map of the human genome. Science 270:1945–1954 (with supplementary data from the Whitehead Institute/MIT Center for Genome Research, Human Genetic Mapping Project, Data Release 12, July 1997).

Ittmann MM, Wieczorek R. 1996. Alterations of the retinoblastoma gene in clinically localized, stage B prostate adenocarcinomas. Hum Pathol 27:28–34.

Kalachikov S, Migliazza A, Cayanis E, Fracchiolla NS, Bonaldo MF, Lawton L, Jelene P, Ye X, Qu X, Chien M, Hauptschein R, Gaidano G, Vitolo U, Saglio G, Resegotti L, Brodjansky V, Yankovsky N, Zhang P, Soares MB, Russo J, Edelman IS, Efstratiadis A, Dalla-Favera R, Fischer SG. 1997. Cloning and gene mapping of the chromosome 13q14 region deleted in chronic lymphocytic leukemia. Genomics 42:369–377.

Kubota Y, Fujinami K, Uemura H, Dobashi Y, Miyamoto H, Iwasaki Y, Kitamura H, Shuin T. 1995. Retinoblastoma gene mutations in primary human prostate cancer. Prostate 27:314–320.

Kunimi K, Bergerheim US, Larsson IL, Ekman P, Collins VP. 1991. Allelotyping of human prostatic adenocarcinoma. Genomics 11:530–536.

Kuroki T, Fujiwara Y, Nakamori S, Imaoka S, Kanematsu T, Nakamura Y. 1995. Evidence for the presence of two tumour-suppressor genes for hepatocellular carcinoma on chromosome 13q. Br J Cancer 72:383–385.

Latil A, Cussenot O, Fournier G, Lidereau R. 1996. The BRCA2 gene is not relevant to sporadic prostate tumours [letter]. Int J Cancer 66:282–283.

Li CD, Larsson C, Futreal A, Lancaster J, Phelan C, Aspenblad U, Sundelin B, Liu Y, Ekman P, Auer G, Bergerheim USR. 1998. Identification of two distinct deleted regions on chromosome 13 in prostate cancer. Oncogene 16:481–487.

Maestro R, Piccinin S, Doglioni C, Gasparotto D, Vukosavljevic T, Sulfaro S, Barzan L, Boiocchi M. 1996. Chromosome 13q deletion mapping in head and neck squamous cell carcinomas: identification of two distinct regions of preferential loss. Cancer Res 56:1146–1150.

Melamed J, Einhorn JM, Ittmann MM. 1997. Allelic loss on chromosome 13q in human prostate carcinoma. Clin Cancer Res 3:1867–1872.

Moskaluk CA, Kern SE. 1997. Microdissection and polymerase chain reaction amplification of genomic DNA from histological tissue sections. Am J Pathol 150:1547–1552.

Nelson JB, Nguyen SH, Zheng H, Ewing CM, Isaacs WB. 1998. A frameshift mutation of the endothelin B receptor gene (EDNRB) in the prostate cancer cell line LNCaP. Proc Am Assoc Cancer Res 39:621.

Nouspikel T, Clarkson SG. 1994. Mutations that disable the DNA repair gene XPG in a xeroderma pigmentosum group G patient. Hum Mol Genet 3:963–967.

Nupponen NN, Hyytinen ER, Kallioniemi AH, Visakorpi T. 1998. Genetic alterations in prostate cancer cell lines detected by comparative genomic hybridization. Cancer Genet Cytogenet 101:53–57.

Pan Y, Matsuyama H, Wang N, Yoshihiro S, Haggarth L, Li C, Tribukait B, Ekman P, Bergerheim US. 1998. Chromosome 16q24 deletion and decreased E-cadherin expression: possible association with metastatic potential in prostate cancer. Prostate 36: 31–38.

Puffenberger EG, Hosoda K, Washington SS, Nakao K, deWit D, Yanagisawa M, Chakravart A. 1994. A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. Cell 79:1257–1266.

Schoenberg M, Cairns P, Brooks JD, Marshall FF, Epstein JI, Isaacs WB, Sidransky D. 1995. Frequent loss of chromosome arms 8p and 13q in collecting duct carcinoma (CDC) of the kidney. Genes Chromosomes Cancer 12:76–80.

Sigurdsson S, Thorlacius S, Tomasson J, Tryggvadottir L, Benediktsdottir K, Eyfjord JE, Jonsson E. 1997. BRCA2 mutation in Icelandic prostate cancer patients. J Mol Med 75:758–761.

Suzuki H, Komiya A, Emi M, Kuramochi H, Shiraishi T, Yatani R, Shimazaki J. 1996. Three distinct commonly deleted regions of chromosome arm 16q in human primary and metastatic prostate cancers. Genes Chromosomes Cancer 17:225–233.

Tamura K, Zhang X, Murakami Y, Hirohashi S, Xu HJ, Hu SX, Benedict WF, Sekiya T. 1997. Deletion of three distinct regions on chromosome 13q in human non-small-cell lung cancer. Int J Cancer 74:45–49.

Terrell RB, Wille AH, Cheville JC, Nystuen AM, Cohen MB, Sheffield VC. 1995. Microsatellite instability in adenocarcinoma of the prostate. Am J Pathol 147:799–805.

Theodoreseu D, Broder SR, Boyd JC, Mills SE, Frierson HF Jr. 1997. p53, bcl-2 and retinoblastoma proteins as long-term prognostic markers in localized carcinoma of the prostate. J Urol 158:131–137.

Toguchida J, McGee TL, Paterson JC, Eagle JR, Tucker S, Yandell DW, Dryja TP. 1993. Complete genomic sequence of the human retinoblastoma susceptibility gene. Genomics 17:535–543.

Trapman J, Sleddens HF, van der Weiden MM, Dinjens WN, Konig JJ, Schroder FH, Faber PW, Bosman FT. 1994. Loss of heterozygosity of chromosome 8 microsatellite loci implicates a candidate tumor suppressor gene between the loci D8S87 and D8S133 in human prostate cancer. Cancer Res 54:6061–6064.

Uchida T, Wada C, Wang C, Ishida H, Egawa S, Yokoyama E, Ohtani H, Koshiba K. 1995. Microsatellite instability in prostate cancer. Oncogene 10:1019–1022.

van den Berg J, Johannsson O, Hakansson S, Olsson H, Borg A. 1996. Allelic loss at chromosome 13q12-q13 is associated with poor prognosis in familial and sporadic breast cancer. Br J Cancer 74:1615–1619.

Visakorpi T, Kallioniemi AH, Syvanen AC, Hyytinen ER, Karhu R, Tammela T, Isola JJ, Kallioniemi OP. 1995. Genetic changes in primary and recurrent prostate cancer by comparative genomic hybridization. Cancer Res 55:342–347.

Watanabe M, Imai H, Shiraishi T, Shimazaki J, Kotake T, Yatani R. 1995. Microsatellite instability in human prostate cancer. Br J Cancer 72:562–564.

Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G. 1995. Identification of the breast cancer susceptibility gene BRCA2. Nature 378:789–792.

Yamaguchi T, Toguchida J, Wadayama B, Kanoe H, Nakayama T, Ishizaki K, Ikenaga M, Kotoura Y, Sasaki MS. 1996. Loss of heterozygosity and tumor suppressor gene mutations in chondrosarcomas. Anticancer Res 16:2009–2015.

Yang-Feng TL, Han H, Chen KC, Li SB, Claus EB, Carcangiu ML, Chambers SK, Chambers JT, Schwartz PE. 1993. Allelic loss in ovarian cancer. Int J Cancer 54:546–551.

The Prostate 41:000–000 (1999)

Loss of Heterozygosity and Lack of Mutations of the *XPG/ERCC5* DNA Repair Gene at 13q33 in Prostate Cancer

Eija-Riitta Hyytinen,[1] Henry F. Frierson Jr.,[1] Tavis W. Sipe,[1] Chang-Ling Li,[2] Armelle Degeorges,[2] Robert A. Sikes,[2] Leland W.K. Chung,[2] and Jin-Tang Dong[1,3]*

[1]*Department of Pathology, University of Virginia Health Sciences Center, Charlottesville, Virginia*

[2]*Department of Urology, University of Virginia Health Sciences Center, Charlottesville, Virginia*

[3]*Department of Biochemistry and Molecular Genetics, University of Virginia Health Sciences Center, Charlottesville, Virginia*

BACKGROUND. Three regions of chromosome 13 were previously identified for having loss of heterozygosity (LOH) in human prostate cancer. One of them, at 13q33, was defined by LOH at markers D13S158 and D13S280. The *XPG/ERCC5* gene, a DNA repair gene that when mutated in the germline leads to xeroderma pigmentosum, has been mapped to 13q33, within one megabase of D13S158 and D13S280. This paper describes LOH and mutational analysis of the *XPG* gene in human prostate cancers, in order to determine whether the *XPG* gene is involved in the development of prostate cancer.

METHODS. LOH of the *XPG* gene was analyzed in 40 primary prostate cancers and 14 metastases by using the microsatellite assay, and its mutations were examined in 5 cell lines, 14 metastases, and 8 tumors with LOH at 13q33 by using the single-strand conformation polymorphism (SSCP)-direct DNA sequencing analysis.

RESULTS. Four of the 29 (14%) informative primary tumors and 4 of 8 (50%) metastases showed LOH for the *XPG* gene. Analysis of the 8 tumors with LOH at the 13q33 region, 14 metastases, and 5 cell lines of prostate cancer revealed two polymorphisms but no mutation of the gene. The polymorphism in exon 2 did not change the amino-acid sequence of the XPG protein, but the exon 15 polymorphism altered codon 1104 from histidine to aspartic acid. The two polymorphisms also occurred in individuals without prostate cancer.

CONCLUSIONS. LOH at *XPG* in prostate cancer supports the conclusion that the 13q33 region contains a gene important in the development of prostate cancer, while lack of mutations of the gene suggests that *XPG* is not the target gene involved. *Prostate 41:00–00, 1999.*

KEY WORDS: **XPG; ERCC5; loss of heterozygosity; gene mutations; prostate cancer**

INTRODUCTION

Complex DNA repair mechanisms are responsible for mending various types of DNA damage that are believed to be responsible for the development of 80–90% of human cancers [1,2]. Excision repair is one of the major pathways of DNA repair, occurring in all free-living organisms [3]. Multiple steps are involved

Grant sponsor: American Cancer Society; Grant number: IRG-149L; Grant sponsor: Research and Development Committee of the University of Virginia School of Medicine; Grant sponsor: US Army; Grant number: DAMD 17-98-1-8636.

*Correspondence to: Jin-Tang Dong, Ph.D., Department of Pathology, University of Virginia Health Sciences Center, Box 214, Charlottesville, VA 22908. E-mail: JD4Q@VIRGINIA.EDU

Received 3 March 1999; Accepted 26 May 1999

in excision repair, that include recognition and removal of damaged bases or nucleotides, gap filling, and ligation [3]. Each of the steps requires different enzymes to accomplish, and defects in the enzymes in humans may result in the diseases of xeroderma pigmentosum (XP), Cockayne's syndrome, and trichothiodystrophy [4]. Patients with XP have an extreme sensitivity to solar ultraviolet (UV) light and are at high risk of developing skin cancer. XP is categorized into seven different groups (A–G), each representing a distinct defective gene in excision repair [4,5].

The XP group G (*XPG*, also called *ERCC5* for excision repair cross-complementing group 5) gene encodes a DNA endonuclease which functions to remove the damaged DNA region during the process of excision repair [6–9]. Mutations of *XPG* have been detected in patients with XP group G and in those with Cockayne's syndrome [10–12]. Cells from patients with XPG are deficient in repairing UV-induced DNA damage, and expression of the wild-type *XPG* gene in these cells restores their DNA repair capacity [7,13,14]. The *XPG* gene has been cytogenetically mapped to the q33 region of chromosome 13 by fluorescence in situ hybridization [15]. Physically, *XPG* is located near markers D13S158 and D13S280, because 1-megabase DNA fragment contains both these markers and the *XPG* gene [16,17]. We demonstrated previously that the 13q33 region involving markers D13S158 and D13S280 has frequent loss of heterozygosity (LOH) in human prostate cancer, suggesting that a gene in the 13q33 region is inactivated during the development of prostate cancer [18]. It is unknown, however, whether *XPG* shows LOH and/or is mutated in prostate cancer.

To determine whether *XPG* is the target gene at 13q33 involved in prostate cancer, we analyzed LOH and mutations of the *XPG* gene in both primary tumors and metastases of human prostate cancer by using polymerase chain reaction (PCR)-microsatellite analysis and the PCR-single-strand conformation polymorphism (SSCP) method. We detected LOH but no mutation of the *XPG* gene in the cases analyzed.

MATERIALS AND METHODS

Tumors and Cell Lines

Forty pairs of zinc formalin-fixed, paraffin-embedded nonneoplastic tissues and prostate cancer specimens from radical prostatectomy specimens from previously untreated patients were used in this study. Patient age ranged from 42–74 years (median, 60). The pathologic characteristics of the tumors were as follows: Gleason score, 82% had scores of 5–7, while 18% had score 8 or 9; lymph node metastasis, 1 of 40 cases; seminal vesicle invasion, 83% negative and 17% positive; and capsule penetration, 64% negative and 36% positive. The tumors were pathologically staged according to the most recent American Joint Committee on Cancer recommendation [19]. Two thirds were pT2 and one third were pT3 cancers. In addition, 14 metastatic cancer specimens from lymph nodes, liver, or bone and matched nonneoplastic tissues from patients who had died of prostate cancer were obtained at autopsy. Tumor cells were collected from 7 μm (hematoxylin and eosin (H and E)-stained sections, using a previously described protocol for preparation of histological sections on glass slides prior to dissection [20]. Using a microscopic dissection method, tumor samples were selected to ensure a minimum of 70% neoplastic cells. Normal cells from lymph nodes or seminal vesicles (or spleen for autopsy specimens) in almost 90% of the cases, and from nonneoplastic prostate in the remainder, were obtained from paraffin blocks that contained no cancer cells. Five human prostate cancer cell lines were also analyzed for *XPG* alterations. Cell lines LNCaP, PC-3, DU-145, and TSU-Pr1 were purchased from the ATCC (Manassas, VA) and were propagated following ATCC instructions. DNA from the ARCaP cell line was provided by Dr. Haiyen Zhau of the University of Virginia Health Sciences Center [21].

LOH Analysis

DNA was isolated from specimens and cells by adding proteinase K solution, incubating at 55°C for 2–3 days, extracting with phenol and chloroform, and precipitating with ethanol. The *XPG* microsatellite marker, which was identified from intron 8 of the human *XPG* gene [15], was synthesized by Life Technologies (Gaithersburg, MD) and used to analyze allelic status of the gene. Each PCR analysis was performed on 10 ng of genomic DNA, using 1 × PCR buffer (1.5 mM $MgCl_2$); 0.4 μM of each primer; 2 μM of each dNTP; 2.5 μCi of α-^{33}P-dATP (3,000 Ci/mmol) (ICN, Irvine, CA); and 0.5 units of Taq DNA polymerase in a volume of 10 μl. Thirty-five cycles, each consisting of denaturation at 94°C for 30 sec, annealing at 51°C for 30 sec, and extension at 72°C for 1 min, were performed. PCR products were separated in a 6% denaturing polyacrylamide gel and autoradiographed for 1–3 days. LOH was determined when the signal for one allele in the tumor was reduced significantly when compared to that for the nonneoplastic cells.

PCR Primers for Mutation Analysis

DNA sequences of exons 1 and 2 and their adjacent intron sequences to amplify these two exons (Table I).

of the XPG gene were available [22], and PCR primers were designed from the intron

TABLE I. Primer Sequences From 5′ to 3′ (5′ Primer/3′ Primer), Annealing Temperatures, and PCR Product Sizes Used for PCR Amplification of the *XPG/ERCC5* Gene From Human Genomic DNA*

Exon no.	5′ primer/3′ primer	Temp (°C)	bp
1	GGTGCAGTCCGTCGTAGAAG/AAGTCCCAACGCGGCGTTAA	55	182
2	AACAAGAGTTCAACTAAAAG/AAACTATACGACACTTACCA	50	382
6	GAAGAGTTCTTTCATAATCCTCAAG/CCTCTGGCATTGCTTCAAATAATG	50	144
7	GAGTCTGATGACTTTTCACAGTACC/ATCAAGATGTAATGTGAAGTGTCT	50	203
8.1	GTATTCAAGCTAAGACAGTTGCAG/CAGCCGATCTTTTCTGTCCT	55	623
8.2	TGCAGAGGAGCACGTAGCCA/CATCAGATTCACTTTCTTCCGAG	55	596
9	GAAGTTTCATTGAAGTGCAAAGTGTG/ATATCTTGCCATTCATGGAGCG	55	238
11	GAACTCCTGCGCCTGTTCGG/CCAATTGATTGTGAAAGTCCACAT	55	214
13	AGAATGGTGGCATGAAGCTCAA/CTCTAATTTTGTCGAGATCAGGT	50	200
15.1	ACACAGCTCCGAATTGATTCCTTC/TCAGCATGTTCACTTGAAGA	50	414
15.2	GGGGAGAGACCTGCCTGCCTCTCAGA/TTACGTCTTTGCGACAAATTCATT	55	382

*Temp, temperature; bp, PCR product sizes in base pairs.

Exon/intron boundaries for the remaining exons of *XPG* were inferred by comparing human *XPG* cDNA (GenBank accession no. X69978) to mouse *XPG* cDNA (GenBank accession no. D16306) and the mouse genomic sequences [23]. The cDNA sequences of human and mouse *XPG* were compared and aligned using the GAP function of the Wisconsin Package version 9.1, Genetics Computer Group (GCG) (Madison, WI). The nucleotides at each of the exon junctions of mouse *XPG* were identical between human and mouse *XPG* genes; therefore, the same junctions were applied to human *XPG* to divide the cDNA into 16 exon fragments. PCR primers were designed from sequences at both ends of each *XPG* exon, and were used to amplify human *XPG* exons from genomic DNA. Two pairs of primers were designed for exons 8 and 15 due to their large sizes. Exons 3–5, 10, 12, and 14, which constitute 13% of the human *XPG* cDNA, were very small and were not analyzed for *XPG* mutations. Primer sequences, annealing temperature, and PCR product size for *XPG* exons are listed in Table I. All primers were synthesized by Life Technologies.

PCR-Single-Strand Conformation Polymorphism (SSCP) Analysis

PCRs for the SSCP analysis were performed in two rounds. The first-round reaction contained 5–10 ng of genomic DNA, 1 × PCR buffer, 0.4 μM of each primer, 200 μM of dATP, dCTP, dGTP, and dTTP, and 0.5 units of Taq DNA polymerase; incubation was for 35 cycles of 94°C for 30 sec, 50–55°C for 30 sec, and 72°C for 1 min. The PCR products were diluted threefold, and 1 μl of the dilution was used as the template for the second-round PCR, which was performed under the same conditions as in the first round PCR except that 2 μM of each dNTP, 2.5 μCi of α-^{33}P-dATP (3,000 Ci/mmol), and 20 cycles were used. The ^{33}P-labeled PCR products were electrophoresed at 5 W overnight at room temperature in a 6% nondenaturing polyacrylamide gel in 1 × TPE buffer (pH 6.8), as described previously [24]. PCR products for exons 2 and 15 were also analyzed in a 0.25 × MDE gel (FMC BioProducts, Rockland, ME) containing 10% glycerol, which were also run at 5 W overnight at room temperature. After electrophoresis, the gels were dried and exposed to Kodak (Rochester, NY) BioMax MR film for 1–2 days. Wild-type and mutant exon 2 of the *PTEN* gene [25] was amplified from normal human DNA and LNCaP DNA, respectively, and the PCR products were run as positive controls along with samples to indicate that sequence variation at a single base in a DNA fragment could be detected in the SSCP gels. The PCR-SSCP procedures were repeated from genomic DNA for samples which showed a band shift, in order to exclude the possibility of PCR artifact.

DNA Sequencing

For the samples which had a band shift in the SSCP analysis, their first-round PCR products were amplified in a volume of 100 μl under the same conditions as the first-round PCR. These PCR products were purified by using the Wizard PCR Preps DNA Purification System (Promega, Madison, WI), and were sequenced by using the TaqTrack Sequencing System (Promega), following the manufacturer's instructions. Sequencing data were collected and analyzed by using ScanDNASIS and MacDNASIS software (Hitachi Software, San Bruno, CA).

RESULTS

LOH at the *XPG* Locus

The *XPG* microsatellite marker was analyzed in 40 primary tumors and 14 metastases of prostate cancer

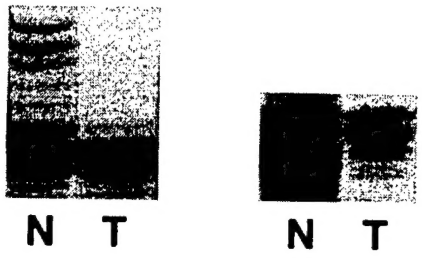

Fig. 1. Loss of heterozygosity of the *XPG/ERCC5* gene in two cases of human prostate cancer, as detected by a microsatellite marker from intron 8 of the gene. N, matched nonneoplastic DNA; T, tumor DNA.

for LOH. Four of the 29 (14%) informative primary tumors showed LOH at the *XPG* locus. In the metastases, 4 of 8 (50%) informative cases had LOH. Examples of LOH in two cases are shown in Figure 1.

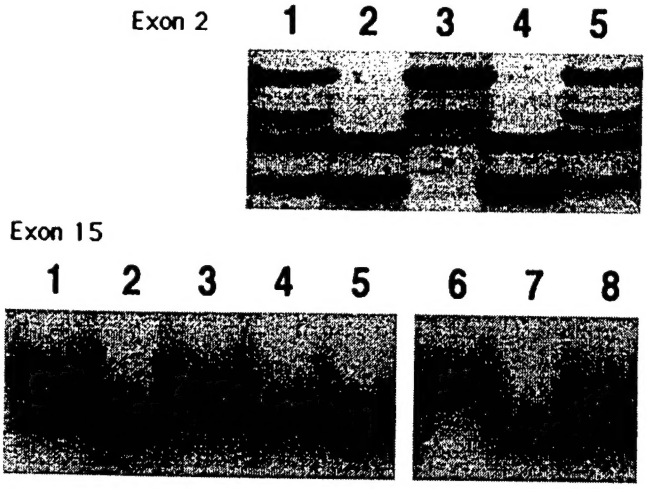

Fig. 2. Different band patterns of polymorphisms for exons 2 and 15 of the *XPG/ERCC5* gene detected by PCR-SSCP analysis. **Lanes 1–5,** cell lines LNCaP, PC-3, ARCaP, DU-145, and TSU-Pr1, respectively; **lanes 6–8,** three tumor cases.

SSCP Analysis

In order to determine whether the *XPG* gene was mutated in prostate cancer, we analyzed the 4 primary tumors which showed LOH at the *XPG* locus, 4 additional primary tumors which showed LOH at *XPG*-linked loci D13S158 and D13S280 [18], 14 metastases of prostate cancer, and 5 cell lines derived from prostate cancer metastases for mutations in nine exons of *XPG* by using PCR-SSCP and direct DNA sequencing. The nine exons analyzed constituted approximately 90% of the coding region of the *XPG* gene. SSCP analysis demonstrated that exons 2 and 15 of *XPG* had variant SSCP bands, suggesting that these two exons had sequence alterations. Matched nonneoplastic cells for each tumor were then analyzed using the same procedure, and all but one (case 50) sample showed the same sizes as did the corresponding tumors. In case 50, the nonneoplastic tissue showed two patterns of bands for exon 15, while the tumor showed only one of the patterns (Fig. 2). These results indicate that different sizes of SSCP bands for exons 2 and 15 represent polymorphisms of the gene, and that case 50 had an LOH of *XPG* in the tumor. In the 14 tumors whose DNA was successfully amplified for exon 2, 6 cases (43%) showed heterozygosity, 6 cases (43%) showed homozygosity for the smaller-band allele, and 2 (14%) showed homozygosity for the larger-band allele (Fig. 2). Similarly for exon 15, 5 of 15 cases (33%) showed heterozygosity, 9 (60%) showed homozygosity for the smaller-band allele, and 1 (7%) showed homozygosity for the larger-band allele (Fig. 2). To verify that the polymorphisms in exons 2 and 15 also occur in normal individuals and to determine the frequencies of these polymorphisms, we analyzed 27 DNA samples from unrelated healthy individuals for the two exons. For exon 2, 7 individuals (26%) demonstrated a heterozygous phenotype, 9 (33%) were homozygous for the smaller-band allele, and 11 (41%) were homozygous for the larger-band allele. For exon 15, the corresponding allelic frequencies in normal healthy individuals were 37% (10 individuals), 59% (16 individuals), and 4% (1 individual).

Sequencing of Variant SSCP Bands

We determined the DNA sequence of the variant bands detected for exons 2 and 15 in tissues and cell lines. Exon 2 was amplified from the five cell lines, since each of the cell lines contained either the upper allele or the bottom allele or both. Direct sequencing of the PCR products revealed a sequence variation at nucleotide 335 (codon 46) of the *XPG* gene (GenBank accession no. X69978), with a thymine (T) in the allele showing larger bands in SSCP analysis and a cytosine (C) in the allele showing smaller band. ARCaP showed a T at this position, as in the reported sequence (GenBank accession no. X69978), while PC-3 and DU-145 had a C, and LNCaP and TSU-Pr1 showed both C and T (Fig. 3). Similarly, exon 15 was also reamplified and sequenced, and a C→G sequence change at nucleotide 3508 of the *XPG* gene was identified (Fig. 3). This alteration occurred in codon 1104 and resulted in a His→Asp amino-acid substitution. The allele showing larger bands in SSCP analysis contained a C, and the allele showing smaller bands contained a G. Since DNAs from healthy individuals showed the same band shifts in SSCP analysis, we also amplified and sequenced some of the normal DNA. Homozygosity for C or G and heterozygosity of C/G

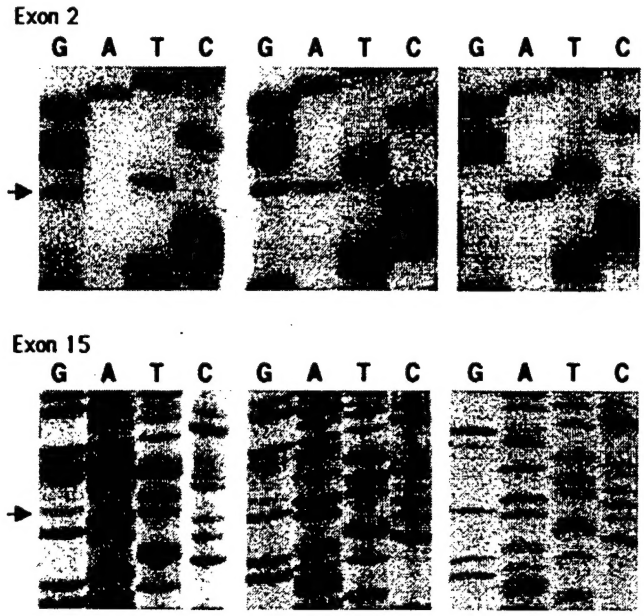

Fig. 3. Sequence analysis of exons 2 and 15 of the *XPG/ERCC5* gene, as primed by reverse primers. Arrows indicate the nucleotide position where polymorphisms occurred. **Left:** Homozygosity for one allele. **Center:** Heterozygosity for two alleles. **Right:** Homozygosity for the other allele.

were found in all the analyzed samples. In case 50, nonneoplastic cells showed heterozygous C/G alleles, but the corresponding tumor cells showed only the G allele, indicating that LOH had occurred.

DISCUSSION

Deletion of chromosome 13 is one of the most frequent genetic alterations in prostate cancer. In this study, we analyzed 62 prostate cancer specimens and cell lines for LOH and mutational status of the *XPG* DNA repair gene, which is located in a previously identified region of LOH at chromosome 13q33 [18]. Using a microsatellite marker isolated from the eighth intron of the gene [15], LOH was found in 14% of the informative primary prostate cancers and 50% of the informative metastases. These LOH frequencies are comparable to those found for the polymorphic markers defining the q33 region (i.e., D13S158 and D13S280) in our previous study [18], that had LOH in 9–14% of the primary tumors and 30% of metastatic tumors. Combining the current study with the previous one [18], 8 tumors had LOH at one or more of the 3 loci in 39 primary tumors which were informative for at least one of the loci, i.e., 8 of 39 (21%) primary tumors had LOH at the 13q33 LOH region. Similarly for the metastases, 7 of 13 (54%) had LOH at this region. Previous studies from other investigators also demonstrated loss at chromosome 13 in prostate cancer and in other malignancies. For example, comparative genomic hybridization revealed loss of 13q in prostate cancer [26–28], and LOH of 13q33 has occurred in ovarian cancer and in head and neck carcinoma [29,30]. These results support the conclusion that there may be a gene located in 13q33 which is important in the development of prostate cancer and some other cancers. However, the target gene in this region has not been identified.

Among the tumors with LOH for any of the three markers (XPG, D13S158, and D13S280), one case had LOH for both XPG and D13S280, and one case had LOH for both D13S280 and D13S158 but not for XPG. Considering that D13S280 is telomeric to D13S158 and both markers are present in the same 1-mb DNA which contains the *XPG* gene [16,17], this finding suggests that the order of the three markers from centromere to telomere is D13S158→D13S280→XPG, and that the 13q33 LOH region likely resides between D13S280 and XPG. Therefore, it is possible that the *XPG* gene is not the target gene at the 13q33 region.

To further determine whether *XPG* plays a role in the development of prostate cancer, we analyzed almost 90% of its coding region in 8 primary tumors with LOH for at least one of the three markers, 14 metastases, and 5 prostate cancer cell lines, using SSCP and direct sequencing methods. No somatic mutation of the gene was detected in any of the samples. This result indicates that mutation of *XPG* is an infrequent event in human prostate cancer, despite the fact that *XPG* is a DNA repair gene and its mutation causes skin cancer in xeroderma pigmentosum [4]. As D13S280 appears to be closer to the center of the 13q33 LOH region relative to XPG and D13S158, this finding also supports the idea that the target gene in the 13q33 LOH region may be located between D13S280 and XPG rather than at the XPG locus.

In our analysis for *XPG* mutations, two polymorphisms, one in exon 2 and the other in exon 15, were identified. The exon 2 polymorphism did not change the amino-acid sequence of the XPG protein, but the exon 15 polymorphism, which occurred at nucleotide position 3508 of the *XPG* gene [13], changed codon 1104 from histidine in one allele to aspartic acid in the other. It is unknown, however, whether this polymorphism affects the repair capacity and/or fidelity of the *XPG* gene and whether it may contribute to the development of prostate cancer. Others have suggested that variation in DNA repair is a factor in cancer susceptibility [31,32]. It might be interesting to analyze the frequencies of the exon 15 polymorphism in larger populations of individuals with and without prostate cancer. We noticed that these polymorphisms were also detected in another study [12].

In conclusion, LOH of the *XPG* gene occurs in hu-

(American Joint Committee on Cancer)

man prostate cancer, but no mutations of this gene could be detected in this study, suggesting that the *XPG* gene is not involved in human prostate cancer and that the target gene at the 13q33 LOH region remains to be identified.

REFERENCES

1. Bohr VA, Phillips DH, Hanawalt PC. Heterogeneous DNA damage and repair in the mammalian genome. Cancer Res 1987;47: 6426–6436.
2. Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst 1981;66:1191–1308.
3. Sancar A. DNA excision repair. Annu Rev Biochem 1996;65:43–81.
4. Bootsma D, Kraemer KH, Cleaver JE, Hoeijmakers JHJ. Nucleotide excision repair syndromes: xeroderma pigmentaosum, cockayne syndrome, and trichothiodystrophy. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 1998. p 245–273.
5. Cleaver JE. Defective repair replication of DNA in xeroderma pigmentosum. Nature 1968;218:652–656.
6. Habraken Y, Sung P, Prakash L, Prakash S. Human xeroderma pigmentosum group G gene encodes a DNA endonuclease. Nucleic Acids Res 1994;22:3312–3316.
7. O'Donovan A, Wood RD. Identical defects in DNA repair in xeroderma pigmentosum group G and rodent ERCC group 5. Nature 1993;363:185–188.
8. O'Donovan A, Davies AA, Moggs JG, West SC, Wood RD. XPG endonuclease makes the 3′ incision in human DNA nucleotide excision repair. Nature 1994;371:432–435.
9. Cloud KG, Shen B, Strniste GF, Park MS. XPG protein has a structure-specific endonuclease activity. Mutat Res 1995;347:55–60.
10. Nouspikel T, Clarkson SG. Mutations that disable the DNA repair gene XPG in a xeroderma pigmentosum group G patient. Hum Mol Genet 1994;3:963–967.
11. Nouspikel T, Lalle P, Leadon SA, Cooper PK, Clarkson SG. A common mutational pattern in Cockayne syndrome patients from xeroderma pigmentosum group G: implications for a second XPG function. Proc Natl Acad Sci USA 1997;94:3116–3121.
12. Okinaka RT, Perez-Castro AV, Sena A, Laubscher K, Strniste GF, Park MS, Hernandez R, MacInnes MA, Kraemer KH. Heritable genetic alterations in a xeroderma pigmentosum group G/Cockayne syndrome pedigree. Mutat Res 1997;385:107–114.
13. Scherly D, Nouspikel T, Corlet J, Ucla C, Bairoch A, Clarkson SG. Complementation of the DNA repair defect in xeroderma pigmentosum group G cells by a human cDNA related to yeast RAD2. Nature 1993;363:182–185
14. Shiomi T, Harada Y, Satio T, Shiomi N, Okuno Y, Yamaizumi M. An ERCC5 gene with homology to yeast RAD2 is involved in group G xeroderma pigmentosum. Mutat Res 1994;314:167–175.
15. Samec S, Clarkson SG, Blaschak J, Chakravarti A, Morris MA, Scherly D, Antonarakis SE. Dinucleotide repeat polymorphism within ERCC5 gene. Hum Mol Genet 1994;3:214.
16. Hudson TJ, Stein LD, Gerety SS, Ma J, Castle AB, Silva J, Slonim DK, Baptista R, Kruglyak L, Xu SH, Hu X, Colbert AME, Rosenberg C, Reeve-Daly MP, Rozen S, Hui L, Wu X, Vestergaard C, Wilson KM, Bae JS, Maitra S, Ganiatsas S, Evans CA, DeAngelis MM, Ingalls KA, Nahf RW, Horton LT, Anderson MO, Collymore AJ, Ye W, Kouyoumjian V, Zemsteva IS, Tam J, Devine R, Courtney DF, Renaud TM, Nguyen H, O'Connor TJ, Fizames C, Faure S, Gyapay G, Dib C, Morissette J, Orlin JB, Birren BW, Goodman N, Weissenbach J, Hawkins TL, Foote S, Page DC, Lander ES. An STS-based map of the human genome. Science 1995;270:1945–1954 (with supplementary data from the Whitehead Institute/MIT Center for Genome Research, Human Genetic Mapping project, data release 12, July 1997).
17. Cayanis E, Russo JJ, Kalachikov S, Ye X, Park SH, Sunjevaric I, Bonaldo MF, Lawton L, Venkatraj VS, Schon E, Soares MB, Rothstein R, Warburton D, Edelman IS, Zhang P, Efstratiadis A, Fischer SG. High-resolution YAC-cosmid-STS map of human chromosome 13. Genomics 1998;47:26–43.
18. Hyytinen ER, Frierson HF, Boyd JC, Chung LWK, Dong JT. Three distinct regions of allelic loss at 13q14, 13q21–22, and 13q33 in prostate cancer. Gene Chromosome Cancer 1999; (in press) 25:105–114.
19. AJCC. AJCC cancer staging manual. 5th ed. Philadelphia: Lippincott-Raven; 1997. p. 294
20. Moskaluk CA, Kern SE. Microdissection and polymerase chain reaction amplification of genomic DNA from histological tissue sections. Am J Pathol 1997;150:1547–1552.
21. Zhau HY, Chang SM, Chen BQ, Wang Y, Zhang H, Kao C, Sang QA, Pathak SJ, Chung LW. Androgen-repressed phenotype in human prostate cancer. Proc Natl Acad Sci USA 1996;93:15152–15157.
22. Samec S, Jones TA, Corlet J, Scherly D, Sheer D, Wood RD, Clarkson SG. The human gene for xeroderma pigmentosum complementation group C (XPG) maps to 13q33 by fluorescence in situ hybridization. Genomics 1994;21:283–285.
23. Ludwig DL, Mudgett JS, Park MS, Perez-Castro AV, MacInnes MA. Molecular cloning and structural analysis of the functional mouse genomic XPG gene. Mamm Genome 1996;7:644–649.
24. Kukita Y, Tahira T, Sommer SS, Hayashi K. SSCP analysis of long DNA fragments in low pH gel. Hum Mutat 1997;10:400–407.
25. Dong JT, Sipe TW, Hyytinen ER, Li CL, Heise C, McClintock DE, Grant CD, Chung LW, Frierson HF Jr. PTEN/MMAC1 is infrequently mutated in pT2 and pT3 carcinomas of the prostate. Oncogene 1998;17:1979–1982.
26. Hyytinen ER, Thalmann GN, Zhau HE, Karhu R, Kallioniemi OP, Chung LWK, Visakorpi T. Genetic changes associated with the acquisition of androgen-independent growth, tumorigenicity and metastatic potential in a prostate cancer model. Br J Cancer 1997;75:190–195.
27. Nupponen NN, Kakkola L, Koivisto P, Visakorpi T. Genetic alterations in hormone-refractory recurrent prostate carcinomas. Am J Pathol 1998;153:141–148.
28. Nupponen NN, Hyytinen ER, Kallioniemi AH, Visakorpi T. Genetic alterations in prostate cancer cell lines detected by comparative genomic hybridization. Cancer Genet Cytogenet 1998; 101:53–57.
29. Maestro R, Piccinin S, Doglioni C, Gasparotto D, Vukosavljevic T, Sulfaro S, Barzan L, Boiocchi M. Chromosome 13q deletion mapping in head and neck squamous cell carcinomas: identification of two distinct regions of preferential loss. Cancer Res 1996;56:1146–1150.
30. Yang-Feng TL, Li S, Han H, Schwartz PE. Frequent loss of heterozygosity on chromosomes Xp and 13q in human ovarian cancer. Int J Cancer 1992;52:575–580.
31. Mohrenweiser HW, Jones IM. Variation in DNA repair is a factor in cancer susceptibility: a paradigm for the promises and perils of individual and population risk estimation? Mutat Res 1998;400:15–24.
32. Shen MR, Jones IM, Mohrenweiser H. Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. Cancer Res 1998;58:604–608.